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HIBERNAL PHENOLOGY OF THE EASTERN BOX  
TURTLE, *TERRAPENE CAROLINA CAROLINA*

BY

MARGARETE WALDEN

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE  
REQUIREMENTS FOR THE DEGREE OF  
MASTER OF SCIENCE

IN

BIOLOGICAL AND ENVIRONMENTAL SCIENCES

UNIVERSITY OF RHODE ISLAND

2017

MASTER OF SCIENCE  
OF  
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2017

## ABSTRACT

The eastern box turtle, *Terrapene carolina carolina*, is a terrestrial ectotherm that is vulnerable due to sustained population declines across its range in the Eastern United States. Where this species uses managed fields, conservation measures could be implemented to restrict field mowing until the local population enters into hibernation, and such mowing restrictions could be adjusted each year only if the precise timing of the entry into and emergence from hibernation could be predicted based on proximate environmental conditions. I monitored twenty turtles each winter over two years to determine movement and activity patterns at the William Floyd Estate, a management unit of Fire Island National Seashore in New York, USA. My objectives were to (1) identify environmental variables correlated with the timing of entry into and emergence from hibernation in order to inform local conservation measures relating to the timing of mowing and brush clearing activities, and (2) investigate whether physiological condition explained the broad variation in the timing of individual animals' responses to the same environmental conditions. I determined the timing of entry into and emergence from hibernation using a combination of light sensors and temperature dataloggers and sampled blood from turtles in the second year before and after hibernation in order to measure plasma biochemical profiles. Cooler air temperatures were correlated with increased probability of entry into hibernation for any given week in the fall, while warmer air temperatures increased the probability of emergence from hibernation for any given week in the spring. Physiological condition was correlated with the number of days until immergence into hibernation in the fall. These findings indicate that environmental conditions are proximate cues that

trigger the timing of hibernation and emergence in turtles, and that physiological condition may mediate these triggers by limiting or forcing responses. Based on these results, managers would benefit from using environmental monitoring to adjust the timing of mowing and brush clearing activities in order to take advantage of longer hibernation times during more severe winters or to limit risk to turtles during warmer falls or earlier onset of warming temperatures in the spring.

## ACKNOWLEDGMENTS

I wish to acknowledge my advisor, Dr. Nancy Karraker, for her support, assistance, expert knowledge, and patience lent throughout this research. I extend my thanks to my committee members, Dr. Scott McWilliams and Dr. Gavino Puggioni, for their substantial improvements to the design and analysis of this work.

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Scott Egan's instructions and guidance regarding VHF radio attachment technique for box turtles was invaluable. Study design suggestions and assistance with weather station assembly given by Rich Inman greatly improved efficiency and aided with field logistics. Dr. Russell Burke and Dr. Thomas Pauley generously shared their expert knowledge of *Terrapene carolina*.

The enthusiasm and helpfulness of my lab members, Anne Devan-Song, Priyanka Patel, Scott Buchanan, Alan Hamilton, and Felicia Woods, cannot be overstated. I also express my gratitude for assistance in the field from Gillian Baird, Kerndja Bien-Aime, Charles Calafiore, Sarah Deckel, Mary Grande, Rebeca Linhart, Raymond Marchinkoski, Vianchell Tiburcio, Lee Tirrell, and Frances Vazquez.

## PREFACE

The following thesis chapters are written in Manuscript Format, and are prepared in adherence to the formatting guidelines of their target publications. Chapter 1 is written in *Journal of Wildlife Management* format in anticipation of submission. This paper synthesizes the applications of research findings to the conservation of the eastern box turtle. Chapter 2 is written in *Comparative Biochemistry and Physiology: Part A: Molecular & Integrative Physiology* format in anticipation of submission. This paper summarizes analyses to understand physiological responses that may explain individual variation in the phenology of hibernation of the eastern box turtle in New York, U.S.A. For both chapters, the following exceptions to the indicated manuscript format have been made for readability and internal consistency within this thesis manuscript: (1) chapter title pages have been formatted similarly, (2) line numbers are removed, (3) numbering of figures and tables includes chapter number, (4) lines are double-spaced, and (5) all paragraphs except the first in a section are indented.

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## CHAPTER 1

# ENVIRONMENTAL CUES AFFECTING THE TIMING OF HIBERNATION IN A POPULATION OF EASTERN BOX TURTLES, *TERRAPENE CAROLINA* *CAROLINA*

by

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**ABSTRACT** Hibernation in temperate reptiles confers protection from environmental extremes during winter. For the eastern box turtle, *Terrapene carolina carolina*, hibernation in the soil can also provide protection from mortality caused by blades and tires associated with mowing equipment. We investigated the timing of hibernation in a population of *T. c. carolina* at a historical site where necessary maintenance of fields may conflict with conservation of this vulnerable species. *T. c. carolina* overwhelmingly selected hibernacula in forested habitat, meaning that turtles would be at lowest risk of mowing mortality during winter months. We found that lower weekly air temperatures were positively correlated with the probability of hibernation in any given week in the fall, while warmer weekly air temperatures resulted in increased probability of emergence from hibernation during any given week in the spring. We conclude that with site-specific environmental data collected during the fall and spring, land managers may be able to adjust field maintenance activities on a year-to-year basis in order to protect local *T. c. carolina* populations once site-specific responses to environmental conditions have been elucidated.

**KEY WORDS** *Terrapene carolina*, hibernation, environmental correlates, temperature, burrowing

The physiological processes and behavior of ectotherms are closely tied to the surrounding environment (Gatten 1974, Adams et al. 1989, Penick et al. 2002).

Hibernation is a crucial aspect of the life history of *T. c. carolina*. Two of the main risks to turtles during prolonged terrestrial torpor are desiccation and predation, and these are successfully avoided by burrowing beneath the soil surface (Ultsch 2006). However, despite freeze tolerance being demonstrated in *T. c. carolina* (Costanzo &

Claussen 1990), lethal cold exposure remains a significant cause of episodic mortality for hibernating adults (Neill 1948; Schwartz and Schwartz 1974). In addition, the warmer winters predicted under various climate change scenarios may decrease growth in hatchlings (McCallum et al. 2009) and result in increased metabolism and depletion of energy reserves prior to spring emergence, resulting in starvation and death (Converse et al. 2005).

The IUCN currently lists *T. carolina* as “vulnerable” due to population declines across its range exceeding 30% over the last 50 years (van Dijk 2013). These declines are thought to have been caused primarily by habitat destruction and fragmentation (Dodd 2001; Budischak et al. 2006; Marsack and Swanson 2009), as well as other anthropogenic impacts, such as recreation (Belzer 1997) and changes in hydrology resulting from upstream water control (Hall et al. 1999). Because *T. carolina* is a long-lived species exhibiting negligible senescence (Henry 2003) and low recruitment, adult-stage mortality significantly impacts population viability (Heppell 1998; Nazdrowicz et al. 2008; Currylow et al. 2011). Anthropogenic causes of high levels of adult mortality include vehicular collisions (Dodd et al. 1989) and mowing and field clearing (Dodd 2001; Nazdrowicz et al. 2008; Erb and Jones 2011). Mowing activities impact box turtle populations from direct mortality from the mower’s blades and tires (Erb and Jones 2011). For this reason, burrowing beneath the soil during winter may protect *T. c. carolina* from risk of mortality during mowing, and thus the timing of hibernation could be important for land managers attempting to conserve vulnerable populations.

The timing of hibernation in adult *T. carolina* has been shown to be correlated with environmental variables such as soil temperature and surface temperature, although there is substantial variation in turtle activity patterns between subspecies and even between populations, and several conflicting trends have been reported. For example, Grobman (1990) found that *T. c. triunguis* held in outdoor enclosures in Missouri emerged after five consecutive days of subsurface temperatures of at least 7 °C. In contrast, Claussen et al. (1991) reported no correlation between body temperatures and entry into or exit from hibernation, although while entry into hibernation was not related to the first killing frost, exit from hibernation always preceded the last killing frost. Interestingly, Claussen et al. (1991) found no significant interannual variation in duration of hibernation. Currylow et al. (2013) reported entry dates for *T. c. carolina* of Oct. 14 – 29 for 89% of turtles in Indiana, with decreasing depths of hibernacula and emergence occurring after the point of inversion between surface and deep soil temperatures at the end of February. In addition to these differing relationships between environmental variables and hibernal phenology, it has been hypothesized that soil moisture may affect timing of emergence (Dodd 2001), but this hypothesis has not been investigated in wild populations.

More precise information about the timing of the adult turtles' entry into and exit from hibernation will be beneficial to managers attempting to reconcile competing advantages and disadvantages of the timing and frequency of mowing activities, as well as contribute to our understanding of this significant yet poorly understood aspect of this species' life history. The objectives of this study were to (1) identify whether *T. c. carolina* behavior during winter months placed them at risk of injury or mortality

from mowing activities, and (2) determine which environmental variables were most strongly associated with the timing of hibernation in *T. c. carolina*.

## **STUDY AREA**

Research was conducted at the 250 ha. William Floyd Estate, a management unit of Fire Island National Seashore, located on the south-central coast of Long Island, NY USA. Forested areas consisted mostly of a coastal-oak heath community, although some areas were dominated by eastern red-cedar, while upland fields were classified as *Dactylis glomerata* – *Rumex acetosella* cultivated herbaceous alliance (Klopfer et al. 2002). Elevation ranges from sea level to 5 m. Monitoring of *T. c. carolina* at this site, including permanent marking of individuals, was begun in 1915 by J. T. Nichols, American Museum of Natural History (Nichols 1939), and monitoring programs have continued into the 21st century. As the William Floyd Estate is an historical site, National Park Service staff have a mandate to maintain the site in the character of the time period being depicted and this includes protection and maintenance of the mosaic of fields and forests that make up the estate. In order to protect the *T. c. carolina* population and grassland nesting birds, the National Park Service prohibits mowing of fields from 1 April to 1 November each year.

## **METHODS**

### **Turtle Monitoring**

In the fall of 2014 and 2015, we performed visual encounter surveys throughout the study area to locate 20 adult box turtles > 350 g mass (2014: 11M:9F; 2015: 10M:10F, sex determination followed Dodd 2001). Radio transmitters (RI-2B, Holohil Systems Ltd., Ontario, CAN) were attached to the carapace using marine epoxy so that turtles



could subsequently be relocated over the winter until spring emergence. Shell temperatures were monitored by attaching a miniature temperature datalogger (iButton Thermochron DS1922L, Embedded Data Systems LLC, KY, USA) to the carapace. Dataloggers were programmed to record at 0.5 °C resolution every 40 minutes and were coated in plastic tool dip (Plasti-Dip International, Circle Pines, MN) prior to attachment to prevent water damage. In order to resolve ambiguity around the determination of entry dates using temperature data alone, in the fall of 2015 we additionally attached a miniature light logger (“geolocator” Intigeo F100, Migrate Technology Ltd., Cambridge, UK) to the carapace, and programmed it to record ambient light levels every 5 min. The combined weight of attachments (radio transmitter, iButton, geolocator when applicable, and all epoxy) averaged 4.58% of body mass (range 2.86 – 7.06%).

Turtles were located at least weekly in the fall using radio telemetry until they began to burrow into the soil or remained in the same location beneath the leaf litter for seven consecutive days. In order to match the shell temperature to soil temperatures to determine the timing of spring emergence, a ground stake was installed in the soil at 50 cm – 200 cm distance from the turtle’s hibernaculum. Each ground stake had three temperature dataloggers (iButton Thermochron DS1922L or DS1921G, Embedded Data Systems LLC, KY, USA) set at the soil surface, 7.5 cm beneath the surface, and 15 cm beneath the surface. Each datalogger was held in a waterproof plastic case containing desiccant that was glued to the wooden stake at the appropriate depth. Once the stake was installed in the soil, it was surrounded by a wire mesh cage to deter animal disturbance. Dataloggers were programmed to record at 0.5

°C resolution every 40 minutes and were subsequently downloaded approximately every 50 days.

Weekly monitoring continued until turtles were determined to have entered into hibernation by remaining in the same location for 14 consecutive days. This represented a conservative determination, as Currylow et al. (2013) defined hibernation as the first day after which the turtle remained underground for at least one week. When necessary, ground stakes were relocated and installed so that the soil temperatures were monitored in close proximity to the hibernacula. Relocation of turtles via radiotelemetry was reduced to monthly in January and February, then was increased to weekly beginning in April to confirm spring emergence. Equipment was removed in May from individuals after all turtles had emerged and dispersed from hibernacula.

Midline straight carapace length (MCL) was measured at first capture. Turtles were weighed at first capture, after equipment attachment or removal, and upon first emergence in the spring. Body condition was calculated as the scaled mass index (Peig and Green 2009) using MCL and the body mass measured at first capture in August – October. The scaled mass index standardizes the prediction for an individual's body condition by accounting for the scaling relationship between body mass and body size, whereas a traditional body condition index tends to be biased towards larger individuals because larger individuals will have larger absolute body components (Peig & Green 2009). Burrowing depth from the surface of the soil to the highest point on the carapace was measured to the nearest centimeter between 15 January – 20 February.

### **Determination of timing of immergence**

We chose to quantify the proportion of days per week that turtles remained in the soil in the fall because turtles did not cease above-ground activity immediately after first burrowing was observed. Instead, turtles seemed to gradually spend a greater number of consecutive days in soil refugia over a period of several weeks, with occasional forays above ground, until seeming to cease above ground movements several weeks after initial burrowing. Therefore, in order to capture this decreased activity, we quantified the proportion of days per week that turtles remained in the soil in the fall by first validating maximum daily light levels recorded by the geolocators in the second study period (fall 2015 – spring 2016). A total of 264 direct observations made over the course of this period were used to identify the maximum daily light level recorded when a turtle was confirmed to be in its refugium and not visible above the leaf litter to an observer. This maximum daily light level of a turtle in its refugium (8.8 lux) was substantially lower than the minimum daily light level recorded when a turtle was observed on the surface (61.6 lux). The conservative definition of the maximum daily light level observed during confirmed refugium occupancy was used as the classification threshold to ensure that no surface activity would be incorrectly classified as refugium use.

We used classification trees (randomForest library, Liaw and Wiener 2002) to build a supervised classification of shell temperatures (minimum, maximum, and mean day versus night temperatures and day length were used as variables; a total of 5000 trees were grown, and five variables were sampled at each node split) for each day for all turtles in the second study period for which we had geolocator data ( $N=19$ ).

The resulting classification (3.84% error) was used to predict daily hibernation status using shell temperatures and day lengths for turtles in the first study period (fall 2014 – spring 2015; n=17) and the one turtle in the second study period for which geolocator data were not available.

Full data for all turtles were available beginning 17 days after the fall equinox in 2015; thus, the proportion of days for which turtles were considered to remain below-ground during the week were calculated beginning on the 17<sup>th</sup> day after the fall equinox for each of 10 weeks, ending with the last complete week before the winter solstice. The fall equinox (22 September 2014, 23 September 2015) and winter solstice (21 December 2014 and 2015), were chosen as biologically relevant circannual events for beginning and ending dates of the immergence period.

### **Determination of timing of emergence**

Date of exit from hibernation was determined as the earliest of either of the following: (1) basking temperatures recorded by the shell data logger (shell temperatures several degrees above surface soil temperatures; Currylow et al. 2013), or (2) direct observation of the turtle above the leaf litter and active. In contrast to the gradual decrease in activity observed in the fall, once turtles came to the surface in the spring, they usually dispersed from the overwintering burrow within three days and rarely returned to a soil burrow after the emergence. Therefore, for each turtle, we classified weeks prior to emergence as “hibernating” and weeks including and after the date of exit as “emerged”. We considered the window of emergence to begin with the first week any turtle emerged and to end with the last week any turtle emerged in either year.

## **Statistical analyses**

All analyses were performed using R Statistical Software v. 3.3.2 ([www.r-project.org](http://www.r-project.org), accessed 9 April 2017). We used t-tests to compare mean burrowing depth beneath the soil and duration of hibernation between years. Turtles found deceased after entry into hibernation (n=2 in 2014 – 2015) were excluded from both comparisons, and turtles for which iButton data were not available (n=3 in 2014 – 2015) were excluded from the latter comparison.

Habitat in which hibernacula were located was characterized as field if the turtle was outside of the tree line, otherwise it was considered forest. Available habitat in each category was quantified using ArcGIS Desktop v. 10.2 (Environmental Systems Research Institute, Inc., Redlands, CA, USA), and a test of proportions was performed to determine whether use differed from availability.

To identify candidate environmental variables that may influence the probability of hibernating for 7 consecutive days in any given week in the fall, a principal components analysis (PCA) was performed on the correlation matrix of 23 environmental variables. The first four components explained 93% of the variance (Table 1.1). We selected the following four environmental variables to test in our model based on loadings in the first four components and biological relevance: weekly mean daily air temperature, coefficient of variation of weekly air temperature calculated using the weekly minimum and maximum temperatures, weekly mean daily soil moisture measured just beneath the soil surface, and weekly mean daily solar radiation. We then fitted a generalized marginal model for binomial proportion data using the logit link and a first-order autoregressive correlation structure (library

geepack; Halekoh et al. 2006), with turtle as the clustering factor and week number as the component within clusters to ensure alignment in the cases of missing weeks due to weather station malfunction. Each candidate variable, including the four selected environmental variables, year, MCL, sex, week, and body condition, was tested singly. Variables with  $P > 0.2$  were excluded from model selection. All possible additive combinations of the remaining candidate variables were then tested, and the model with the lowest quasi-likelihood under the independence model criterion (QIC; Pan 2001) value was selected as the final model.

We used a similar approach to determine the influence of the environment on the probability of emergence from hibernation in any particular week in the spring. The first four components from the PCA of 23 environmental variables explained 86.4% of the variance (Table 1.3). Because these loadings were similar to the principal components analysis performed for the fall dataset, and to ensure consistency between seasonal predictions, we chose the same four environmental variables to test in our model. These four variables and the candidate variables of year, MCL, sex, week, and body condition were tested singly in the generalized marginal model with turtle as the clustering factor, and variables with  $P > 0.2$  were excluded from model selection. All possible additive combinations of the remaining candidate variables were then tested. After comparing the candidate models using the independence model criterion, several models were within 2 QIC values of the lowest QIC, so the most parsimonious model of that set was selected as the final model.

## RESULTS

In 2014, all turtles had moved out of fields and into the forest, with no straightline-crossing of fields or roads as determined by weekly GPS locations, as of 2 November (with the exception of one individual who hibernated outside of the tree line at the edge of a field), and in 2015 by 8 November. Earliest entry dates into hibernation were 19 October 2014 and 11 October 2015. One out of 40 turtles hibernated in a field, representing 2.5% of study animals, whereas 14.1 % of available habitat was categorized as “field”. Turtles avoided fields as locations of hibernacula ( $Z=-2.108$ ,  $P=0.0175$ ).

The period over which any monitored turtle was in hibernation (first immergence to last emergence) was 19 October 2014 – 12 May 2015 and 11 October 2015 – 9 May 2016. The period during which all monitored turtles were simultaneously in hibernation (last immergence to first emergence) spanned 11 November 2014 – 6 April 2015 and 16 December 2015 – 11 March 2016. Average duration of hibernation was longer ( $t_{30,0.05} = 3.00$ ,  $P = 0.004$ ) in 2014 – 2015 ( $184 \pm 13$  days, range 156 – 200 days) than in 2015 – 2016 ( $164 \pm 22$  days, range 119 – 203 days). The average depth of hibernation beneath the soil was  $4.3 \pm 2.3$  cm (range 0 – 7.5 cm) in 2014 – 2015, and did not differ ( $t_{38,0.05} = -0.321$ ,  $P = 0.750$ ) from that in 2015 – 2016 ( $4.5 \pm 2.1$  cm, range 1 – 9 cm). Depth of the single turtle that hibernated in a field over the winter of 2014 – 2015 was 4.2 cm

The last confirmed date prior to any turtle moving into a field or across a road as determined from weekly GPS locations and straight-line distances between

observations in the spring was 18 April 2015 and 11 March 2016. Emergence of all turtles from hibernation was documented by 12 May 2015 and 9 May 2016.

Only weekly mean daily temperature was included in the final model for the probability of hibernation during any one week in the fall (Table 1.2). Lower temperatures resulted in higher probability of hibernation in a given week (Figure 1.1). The inflection point in the predicted response curve is at 13.9 °C, meaning that at weekly mean daily temperatures of less than 11.3 – 17.1 °C, the probability of hibernation in any given week is predicted to be at least 50%. At weekly mean daily temperatures of less than 6.3 – 10.8 °C, the probability of hibernation in any given week is predicted to be higher than 90%.

Weekly mean daily temperature and year were included in the final model for the probability of emerging from hibernation in the spring in any given week (Table 1.4). There was a slightly lower intercept for year 2015 than year 2016, although the parameter estimate was not significantly different from zero in the model. (Figure 1.2). The inflection point in the predicted response curve for 2015 is at 11.8°C and for 2016 is at 10.4 °C, meaning that at weekly mean daily temperatures of greater than 7.0 – 15.4 °C, depending on yearly conditions, the probability of emergence from hibernation in any given week is predicted to be at least 50%. The probability of emergence in any given week is predicted to be higher than 10% when weekly mean daily temperature is warmer than 3.0 °C – 10.3 °C, depending on yearly conditions.

The scaling exponent estimated by SMA regression of body mass on MCL was 1.706, and the arithmetic mean value for MCL for the study population was 134.5 mm.



## DISCUSSION

Turtles preferentially selected forest habitat for hibernacula locations. This avoidance of fields, similar to the avoidance of clearcuts in Indiana (Currylow et al. 2013), is likely due to soil compaction and more variable thermal profiles of the different soil types typical in fields or areas without canopy cover; however, such avoidance also translates to lower risk to turtles from late fall, winter, and early spring mowing activities because turtles are unlikely to be present in fields at all. Furthermore, the radio-tracked individuals in this study were observed to decrease weekly distances moved and to cease using field and edge habitat in advance of entry into hibernation. Movement out of fields and into forests, in conjunction with a reduction in overall movements, provides an additional buffer against the risk of mowing-caused mortalities even for turtles that have not yet entered into hibernation after mowing activities resume in the fall.

We determined that weekly mean air temperatures affected the probability of entry into and emergence from hibernation in any given week in the fall or spring, respectively. In the fall, cooler temperatures increased the probability that individuals would either begin burrowing into the soil or remain underground during the week. Warmer temperatures in the spring increased the probability that individuals would emerge from hibernation during the week, supporting findings from previous studies on *T. carolina* (Grobman 1990; Claussen et al. 1991; Currylow et al. 2013). We did not find support for the hypothesis that soil moisture would affect the timing of hibernation. In contrast to the previously mentioned studies, however, turtles spent longer in hibernation in the first year of our study than in the second year. The

northeastern United States experienced a “much below average” temperature ranking for the coastal New York climate division for January-March 2015 when compared with climate normals since 1895 (NCEI 2017). This unusual winter may explain why the average hibernation duration was longer over the 2015 – 2016 winter, and in turn why the response curve for the probability of emergence from hibernation in any given week was steeper for 2016 than 2015 across the temperature gradient.

Such results join the growing body of literature indicating that climatic variation affects seasonal phenology across a range of taxa (Inouye et al. 2000; Parmesan and Yohe 2003). Warming trends in the northeastern US under various climate change scenarios show strong increases in mean winter temperatures (Kunkel et al. 2013). Earlier emergence from hibernation in this population does appear to be related to warmer winter conditions, and as a result an overall trend of earlier spring emergence might be developing, although such a suggestion is purely speculative. If this is the case, however, there may be implications for survivorship. For example, Converse et al. (2005) modeled decreased survival in a population of *Terrapene o. ornata* in Nebraska when minimum winter temperatures were higher, and the authors hypothesized that mortality was due to the depletion of energy reserves as a result of increased metabolism from warmer ambient temperatures. Similarly, McCallum et al. (2009) predicted reduced annual growth rates in *Terrapene c. triunguis* when winter temperatures were warmer, and such reduced growth can impact chelonian fecundity because clutch size is related to female body size. The authors concluded that climate change and warmer temperatures would threaten population persistence. Whether our

study population may be at risk due to decreased survivorship or reduced fecundity from warming trends would require long-term monitoring, however.

## **MANAGEMENT IMPLICATIONS**

Turtles at this unit of Fire Island National Seashore avoided overwintering in fields and had moved away from fields and roads by 2 – 8 November in 2014 and 2015. Our results support the current management regime at this site that limits mowing activities to November – March, so as to coincide with the period of turtle hibernation.

Furthermore, reduced movements in the fall suggest a corresponding reduction of the risk of mowing activities to turtles even though some individuals delayed entry into hibernation until November or December. Because air temperatures affected the probability of entry into and emergence from hibernation in any given week in the fall and spring, respectively, monitoring of temperatures at this site would be informative because climatic variability between years might allow earlier mowing in the fall or later mowing in the spring. As these are necessarily site-specific recommendations, expanding this study to additional sites would require monitoring individual turtles to determine whether other populations exhibit a similar response to environmental conditions. Once the response curve has been established at a site, further monitoring of environmental conditions may be informative for understanding turtle movement out of fields and the timing of hibernation at specific sites. Finally, we recommend long-term monitoring of survival and fecundity rates in order to better assess population trends as climate change accelerates.

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Table 1.1. Principal components analysis of environmental variables for weekly conditions during the fall of 2014 and 2015 at the William Floyd Estate in New York, USA. The first four components explained 93% of the variance. Loadings  $\geq 0.2$  are in bold.

|                                                                    | Comp. 1      | Comp. 2      | Comp. 3     | Comp. 4      |
|--------------------------------------------------------------------|--------------|--------------|-------------|--------------|
| Mean weekly day length                                             | <b>-0.27</b> |              |             | -0.11        |
| Minimum daily minimum air temperature during week                  | <b>-0.23</b> | 0.18         | -0.13       | 0.13         |
| Maximum daily maximum air temperature during week                  | <b>-0.24</b> | <b>-0.20</b> | 0.10        | -0.15        |
| Mean daily mean air temperature during week                        | <b>-0.28</b> |              |             |              |
| Mean daily relative humidity during week                           |              | <b>0.31</b>  |             | <b>0.32</b>  |
| Total weekly precipitation                                         |              | 0.11         | <b>0.56</b> | 0.19         |
| Maximum daily maximum barometric pressure during week              | 0.16         |              | <b>0.17</b> | <b>-0.54</b> |
| Minimum daily minimum barometric pressure during week              |              | <b>0.35</b>  |             | <b>-0.34</b> |
| Mean daily mean barometric pressure during week                    | 0.15         | <b>0.22</b>  | <b>0.15</b> | <b>-0.49</b> |
| Minimum daily minimum soil surface temperature during week         | <b>-0.25</b> | 0.17         |             | 0.16         |
| Maximum daily maximum soil surface temperature during week         | <b>-0.26</b> | -0.13        |             | -0.16        |
| Mean daily mean soil surface temperature during week               | <b>-0.28</b> |              |             |              |
| Minimum daily minimum soil temperature at 7.5 cm depth during week | <b>-0.26</b> | 0.14         |             |              |
| Maximum daily maximum soil temperature at 7.5 cm depth during week | <b>-0.28</b> |              |             | -0.15        |
| Mean daily mean soil temperature at 7.5 cm depth during week       | <b>-0.28</b> |              |             | -0.11        |
| Minimum daily minimum soil temperature at 15 cm depth during week  | <b>-0.25</b> | 0.12         |             |              |
| Maximum daily maximum soil temperature at 15 cm depth during week  | <b>-0.27</b> |              |             | -0.16        |
| Mean daily mean soil temperature at 15 cm depth during week        | <b>-0.27</b> |              |             | -0.12        |
| Mean daily mean soil moisture at 1 cm depth during week            |              | <b>-0.43</b> |             |              |
| Mean daily mean soil moisture at 7.5 cm depth during week          |              | <b>-0.43</b> |             |              |
| Mean daily mean soil moisture at 15 cm depth during week           |              | <b>-0.44</b> |             |              |
| Maximum daily maximum solar radiation during week                  | -0.16        |              | <b>0.49</b> | 0.11         |
| Mean daily mean solar radiation during week                        | -0.10        |              | <b>0.56</b> |              |

Table 1.2. Model selection using QIC to explain probability of hibernation of *Terrapene carolina carolina* for any one week in the fall of 2014 and 2015 in New York, USA, and parameter estimates for the final model. Only the first five candidate models with the lowest QIC are shown. “CoV temperature” is the coefficient of variation of the weekly mean daily temperature.

| No.                          | Model                                    | QIC              | $\Delta$ QIC      | Weight | Quasi-log likelihood |
|------------------------------|------------------------------------------|------------------|-------------------|--------|----------------------|
| 1.                           | mean temperature                         | 252.3            | 0.00              | 0.790  | -114.18              |
| 2.                           | mean temperature + CoV temperature       | 255.0            | 2.66              | 0.209  | -113.14              |
| 3.                           | mean temperature + MCL                   | 267.0            | 14.66             | 0.001  | -112.42              |
| 4.                           | mean temperature + CoV temperature + MCL | 268.1            | 15.72             | 0.00   | -111.21              |
| 5.                           | mean temperature + body condition        | 270.0            | 17.67             | 0.00   | -112.50              |
| Selected model               |                                          | Parameters       | Estimate $\pm$ SE | Wald   | P value              |
| intercept + mean temperature |                                          | intercept        | 5.44 $\pm$ 0.55   | 96.8   | <0.001               |
|                              |                                          | mean temperature | -0.39 $\pm$ 0.04  | 100.9  | <0.001               |

Table 1.3. Principal components analysis of environmental variables for weekly conditions in the spring of 2015 and 2016 at the William Floyd Estate in New York, USA. The first four components explained 86.4% of the variance. Loadings  $\geq 0.2$  are in bold.

|                                                                    | Comp.<br>1   | Comp.<br>2   | Comp.<br>3   | Comp.<br>4   |
|--------------------------------------------------------------------|--------------|--------------|--------------|--------------|
| Mean weekly day length                                             | <b>-0.25</b> |              |              | -0.16        |
| Minimum daily minimum air temperature during week                  | <b>-0.25</b> |              | -0.16        | 0.12         |
| Maximum daily maximum air temperature during week                  | <b>-0.23</b> | -0.15        |              |              |
| Mean daily mean air temperature during week                        | <b>-0.27</b> |              |              | -0.10        |
| Mean daily relative humidity during week                           |              | <b>0.32</b>  | <b>-0.49</b> | -0.14        |
| Total weekly precipitation                                         |              | <b>0.37</b>  | -0.19        | 0.11         |
| Maximum daily maximum barometric pressure during week              | 0.12         |              | <b>-0.52</b> | <b>0.32</b>  |
| Minimum daily minimum barometric pressure during week              |              | <b>-0.32</b> |              | <b>0.28</b>  |
| Mean daily mean barometric pressure during week                    |              | <b>-0.32</b> | <b>-0.34</b> | <b>0.45</b>  |
| Minimum daily minimum soil surface temperature during week         | <b>-0.25</b> |              | <b>-0.20</b> |              |
| Maximum daily maximum soil surface temperature during week         | <b>-0.26</b> |              | 0.17         | 0.18         |
| Mean daily mean soil surface temperature during week               | <b>-0.27</b> |              |              |              |
| Minimum daily minimum soil temperature at 7.5 cm depth during week | <b>-0.27</b> |              |              |              |
| Maximum daily maximum soil temperature at 7.5 cm depth during week | <b>-0.27</b> |              |              |              |
| Mean daily mean soil temperature at 7.5 cm depth during week       | <b>-0.27</b> |              |              |              |
| Minimum daily minimum soil temperature at 15 cm depth during week  | <b>-0.27</b> |              |              |              |
| Maximum daily maximum soil temperature at 15 cm depth during week  | <b>-0.27</b> |              |              |              |
| Mean daily mean soil temperature at 15 cm depth during week        | <b>-0.27</b> |              |              |              |
| Mean daily mean soil moisture at 1 cm depth during week            |              | <b>0.32</b>  | <b>0.36</b>  | <b>0.34</b>  |
| Mean daily mean soil moisture at 7.5 cm depth during week          |              | <b>0.34</b>  |              | <b>-0.26</b> |
| Mean daily mean soil moisture at 15 cm depth during week           |              | <b>0.36</b>  | <b>0.18</b>  | <b>0.49</b>  |
| Maximum daily maximum solar radiation during week                  | <b>-0.20</b> | -0.15        |              | <b>-0.23</b> |
| Mean daily mean solar radiation during week                        | -0.14        | <b>-0.36</b> | <b>0.22</b>  |              |

Table 1.4. Model selection using QIC to explain probability of emergence from hibernation of *Terrapene carolina carolina* for any one week in the spring of 2014 and 2015 in New York, USA, and parameter estimates for the final model. The first 10 models with the lowest QIC scores are shown.

| No.              | Model                                              | QIC        | $\Delta$ QIC      | Weight | Quasi-log likelihood |
|------------------|----------------------------------------------------|------------|-------------------|--------|----------------------|
| 1.               | mean temp + mean solar radiation + CoV temp + year | 240        | 0.00              | 0.266  | -123                 |
| 2.               | mean temp + mean solar radiation + year            | 240        | 0.23              | 0.237  | -123                 |
| 3.               | mean temp + CoV temp + year                        | 241        | 0.86              | 0.173  | -123                 |
| 4.               | mean temp + year                                   | 241        | 1.20              | 0.146  | -124                 |
| 5.               | mean temp + mean solar radiation + CoV temp        | 242        | 2.19              | 0.089  | -125                 |
| 6.               | mean temp + mean solar radiation                   | 242        | 2.46              | 0.078  | -125                 |
| 7.               | mean temp + CoV temp                               | 248        | 8.31              | 0.004  | -128                 |
| 8.               | mean temp                                          | 249        | 8.75              | 0.003  | -128                 |
| 9.               | mean solar radiation + CoV temp + year             | 360        | 119.83            | 0.000  | -183                 |
| 10.              | mean solar radiation + year                        | 364        | 123.69            | 0.000  | -185                 |
| Selected model   |                                                    | Parameters | Estimate $\pm$ SE | Wald   | P value              |
|                  |                                                    | intercept  | -5.78 $\pm$ 0.83  | 49.28  | < 0.001              |
| mean temp + year |                                                    | mean temp  | 0.488 $\pm$ 0.06  | 70.72  | < 0.001              |
|                  |                                                    | year       | 0.685 $\pm$ 0.41  | 2.81   | 0.094                |

Figure 1.1. Probability of hibernation of *Terrapene carolina carolina* in any one week in the fall of 2014 and 2015 in New York, USA, by weekly mean daily temperature, including 95% confidence region.

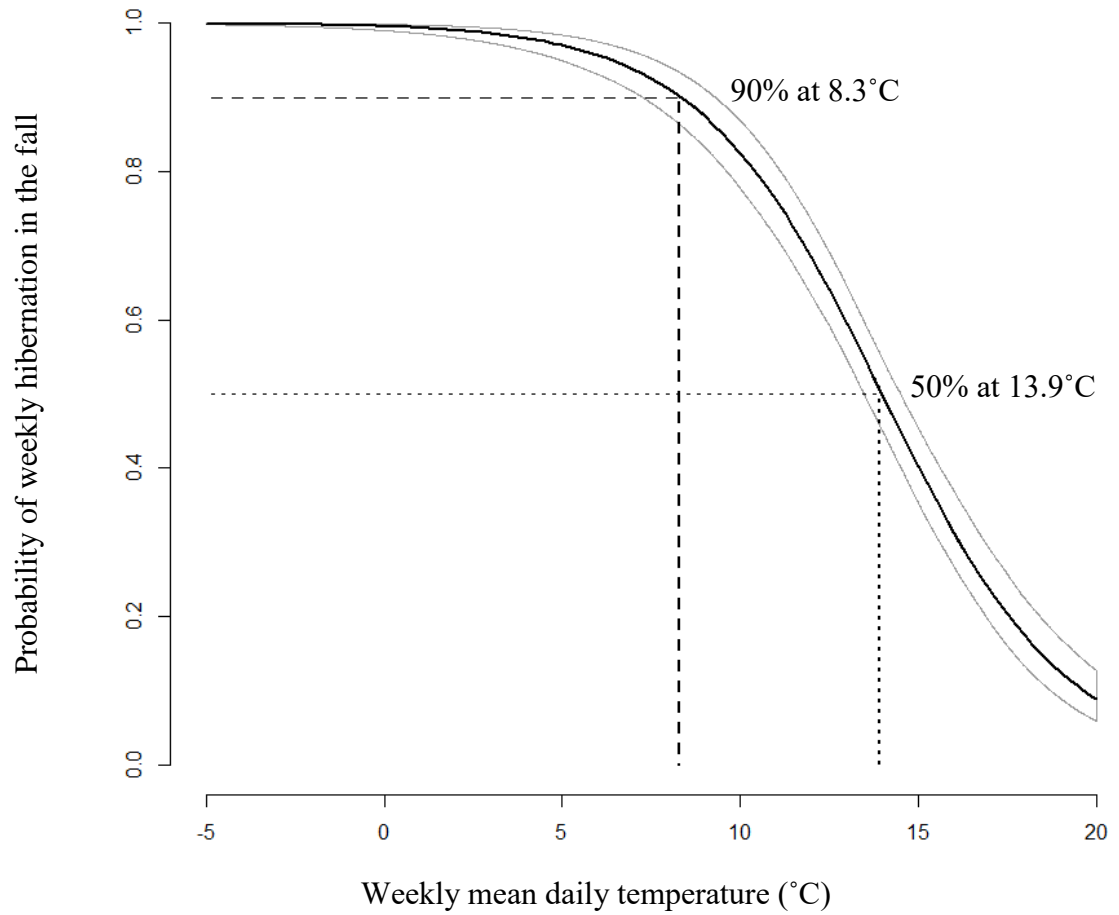
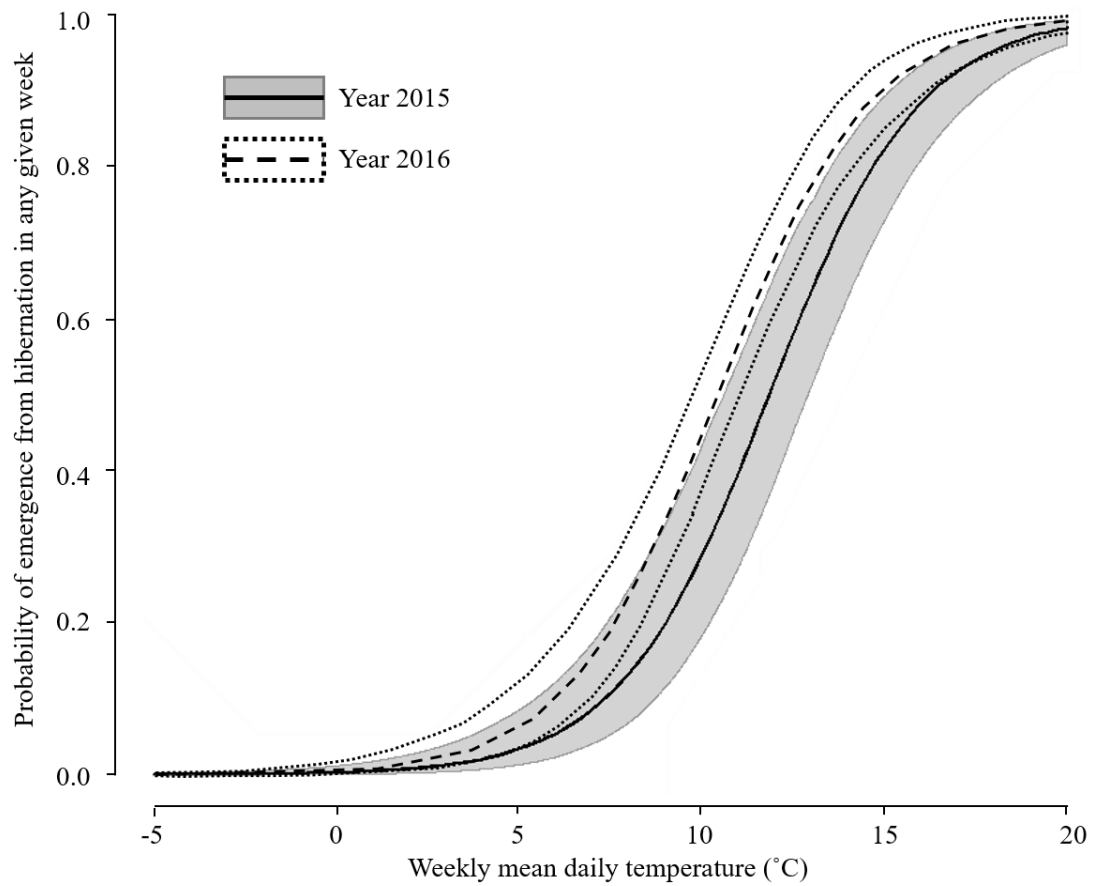


Figure 1.2. Probability of emergence from hibernation of *Terrapene carolina carolina* in any one week in the spring of 2015 and 2016 in New York, USA, by weekly mean daily temperature, including 95% confidence region.



## CHAPTER 2

# DOES PHYSIOLOGICAL CONDITION EXPLAIN ASYNCHRONY IN THE OVERWINTERING PHENOLOGY OF A TERRESTRIAL ECTOTHERM (TESTUDINES: EMYDIDAE)?

by

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## **Abstract**

Intraspecific variation in the timing of overwintering behavior in ectotherms suggests that environmental conditions are not the sole proximate triggers of seasonality within populations. Plasticity in individual responses to environmental triggers of seasonality allow individuals to adapt *in situ* to changing climatic conditions, and such responses may be influenced by physiological condition. We measured body size, body mass, and plasma biochemical profiles as indices of physiological condition before and after hibernation in a population of eastern box turtles (*Terrapene carolina carolina*) in the northern portion of their range. Lower concentrations of sodium and overall solutes, and higher concentrations of potassium and calcium, were associated with fewer days to immergence into hibernation in the fall. We failed to find support for our hypothesis that plasma biochemical profiles would be related to the timing of emergence from hibernation in the spring; however, it may be that relative changes in physiological condition over the duration of hibernation, rather than absolute concentration of solutes measured after emergence, may be more biologically relevant to the timing of emergence. Physiological condition appears to act as a proximate mechanism for determining some aspects of an individual's response to changing environmental conditions in relation to hibernal phenology. This proximate mechanism for behavioral plasticity may enable persistence as climate change accelerates.

**Key words:** asynchrony, hibernation, body condition, plasma biochemistry,

*Terrapene carolina*.



## 1. Introduction

Hibernation is a critical aspect of the life history of terrestrial ectotherms, and the timing of hibernation can influence access to mates, timing of reproduction, depletion of energy reserves, protection from desiccation, exposure to predation, and risk of experiencing lethal cold temperatures (Neill 1948; Carpenter 1957). Endogenous circannual rhythms and exogenous photoperiod have been demonstrated to influence the seasonality of behavior across vertebrate taxa (Helm *et al.* 2013). Ectotherms, because their metabolic rate is dependent upon external temperatures, respond to environmental conditions as proximate cues resulting in year-to-year variation in the timing of immergence and emergence in hibernating populations (Gregory 1982).

Most terrestrially hibernating chelonian species demonstrate a range of intraspecific variation in the timing of immergence and emergence, making the identification of precise thresholds of environmental triggers challenging (Blouin-Demers, Prior & Weatherhead 2000). Explanations for intraspecific variation in hibernal phenology in mammals have included fall body condition, genetic associations, and sex, although, with the exception of sex, such variables have rarely been thoroughly investigated in field studies of ectotherms (Blouin-Demers *et al.* 2000).

This study aimed to examine whether physiological condition could explain individual variation in the timing of hibernation in a population of eastern box turtles, *Terrapene carolina carolina* (Linnaeus 1758), a terrestrial hibernator with a large range across most of the Eastern United States. Previous studies have not found that

sex affected timing of hibernation in wild *T. c. carolina* (Currylow, Macgowan & Williams 2013), but to the authors' knowledge, no study has yet investigated the relationship between indicators of physiological condition and individual dates of hibernation in wild populations of *T. c. carolina*. It is unknown whether *T. c. carolina* attempt to remain in hibernation as long as possible in order to reduce their risk of exposure to freezing temperatures, or if the period of hibernation is reduced as much as possible to enable foraging in the fall or searching for mates in the spring. If the potential cost of the risk of exposure to freezing temperatures is greater than the potential benefits of foraging or mate searching, *T. c. carolina* may adjust the timing of entry into or exit from hibernation according to available energy stores rather than, or in addition to, environmental conditions.

Body condition is a commonly used index that measures muscle and fat stores after correcting for body size. However, the effects of changes in body condition of turtles on individual behavior may not be detectable because the amount of water stored in the bladder can overwhelm subtle changes in tissue mass (McCoy et al. 2011). In addition, the accumulation of fat stores in anticipation of hibernation has not been observed in many turtle species (Ultsch 1989), and Brisbin (1972) did not observe captive *T. c. carolina* utilizing stored fat reserves over the winter. Instead of relying on brown adipose tissue to fuel metabolism during periodic arousals while hibernating, as heterothermic mammals and birds have been observed to do (Dark 2005), turtles generally rely on the storage of carbohydrates in the form of glycogen in order to fuel energy needs over the winter (Hutton & Goodnight 1957; Ultsch 1989). Glycogen is converted to glucose by enzymes in the liver even during winter, and

because muscle tissue and other organs experience lower metabolic rates during this time, glucose accumulates in blood plasma, thus providing a source of immediately available energy upon emergence (Hutton and Goodnight 1957) as well as acting as a cryoprotectant (Costanzo, Lee Jr. & Wright 1993). Thus, the accumulation of glucose in blood plasma measured at emergence from hibernation may be a more accurate indicator of endogenous energy stores and hence physiological condition than body mass.

In addition to plasma glucose, there are many plasma biochemical variables that serve as indicators of various aspects of physiological condition in turtles and which show seasonal patterns of changes in concentration; these candidate variables may affect timing of immergence or emergence. We identified eight variables, including glucose, for which normal reference ranges in *T. c. carolina* have been reported in the literature and that collectively capture major physiological attributes, specifically metabolism, ion balance, and tissue and cell damage. Protein catabolism provides a source of energy for turtles during hibernation in addition to glycolysis (Hutton and Goodnight 1957). The accumulation of uric acid in blood plasma over the winter indicates protein catabolism, and uric acid can also serve as a cryoprotectant (Costanzo and Claussen 1990). Calcium is necessary for egg calcification (Norris and Carr 1996), and is elevated in breeding females (Kimble and Williams 2012). The plasma electrolytes sodium and potassium are routinely measured as part of health assessments, and concentrations of both are depressed in unhealthy animals (Adamovicz et al 2015). Elevated levels of aspartate aminotransferase (AST) in plasma indicate tissue damage in liver or muscle, while elevated levels of lactate

dehydrogenase (LDH) can indicate cryoinjury in overwintering turtles, specifically freeze/thaw damage to plasma membranes (Costanzo, Baker, & Lee 2006). Plasma osmolality provides a measure of hydration status and indicates overall concentration of dissolved solutes in the blood (Ultsch et al. 1999).

We hypothesized that plasma biochemical profiles of *T. c. carolina* consisting of these eight variables, but not body condition, would be correlated with immergence and emergence dates of hibernation. Specifically, we predicted that (1) turtles with better physiological condition in the fall would enter into hibernation earlier, and (2) turtles with better physiological condition in the spring would emerge from hibernation later.

## **2. Materials and methods**

### *2.1 Ethical procedures*

All animal handling procedures were approved by the Institutional Animal Care and Use Committee of the University of Rhode Island (IACUC Protocol #AN-1415), and were permitted under the U.S. National Park Service Scientific Collecting and Research Permits ##FIIS-2013-SCI-0005, #FIIS-2014-SCI-0002, and #FIIS-2015-SCI-0028.

### *2.2 Study area*

Research was conducted at the 250 ha. William Floyd Estate, a management unit of Fire Island National Seashore, located on the southern coast of Long Island, New

York, USA (40° 45'57" N, 72° 49' 26" W). This site occurs in the Long Island Coastal Lowlands section of the Atlantic Coastal Plain physiographic province, and elevation ranges from sea level to 5 m. Forested areas consisted mostly of a coastal-oak heath community, although some areas were dominated by eastern red-cedar, while upland fields were classified as *Dactylis glomerata* – *Rumex acetosella* cultivated herbaceous alliance (Klopfer *et al.* 2002).

### 2.3 Turtle monitoring

In August – September 2015, we performed visual encounter surveys throughout the study area to locate 20 adult box turtles > 350 g mass (10M:10F, sex determination followed Dodd 2001). Radio transmitters (RI-2B 10 - 15 g, Holohil Systems Ltd., Ontario, CAN) were attached to the carapace using marine epoxy so that turtles could subsequently be relocated over the winter until spring emergence. To determine hibernation exit dates, shell temperatures were monitored by attaching a miniature temperature datalogger (iButton Thermochron DS1922L, Embedded Data Systems LLC, KY, USA) to the carapace. Dataloggers were coated in plastic tool dip (Plasti-Dip International, MN, USA) to prevent water damage with minimal effect on accuracy (Roznik & Alford 2012), and were programmed to record at 0.5 °C resolution every 40 minutes. In order to resolve ambiguity around the determination of entry dates using temperature data alone, we additionally attached an archival light logger (“geolocator” Intigeo F100, Migrate Technology Ltd., Cambridge, UK) to the carapace, and programmed it to record maximum ambient light levels (0.28 – 18604

lux) every 5 min. The combined weight of attachments (radio transmitter, iButton, geolocator, and all epoxy) averaged 4.58% of body mass (range 2.86 – 7.06 %).

Turtles were located via radio telemetry at least weekly in the fall until they began to burrow into the soil or remained in the same location beneath the leaf litter for 7 consecutive days. Turtles were operationally defined as having entered into hibernation by remaining in the same location for 14 consecutive days, at which point monitoring via radio telemetry was reduced to monthly until March. We considered 14 days to be a conservative determination, as Currylow et al. (2013) defined entry into hibernation as the first day after which the turtle remained underground for at least one week. Weekly monitoring resumed in April to confirm spring emergence. Equipment was removed in May after all turtles had emerged and dispersed from hibernacula.

#### *2.4 Quantifying body condition*

Midline straight carapace length (MCL) was measured at first capture. Turtles were weighed to the nearest 5 g using a Pesola hanging scale at first capture, before and after equipment attachment or removal, at blood sampling, and upon first emergence in the spring. Mass measurements on each sampling occasion were corrected by removing the mass of attached equipment from total mass.

#### *2.5 Blood sampling procedure*

In October 2015, 0.5 ml of whole blood was collected via venipuncture of the dorsal subcarapacial cervical plexus (Hernandez-Divers, Hernandez-Divers & Wyneken 2002) in the first week, and subsequently every two weeks until the turtle

was operationally determined to have entered into hibernation. A total of between one to three samples were collected from each individual, as monitoring visits occurred weekly and no individuals were observed aboveground during monitoring visits after October 30, 2015. In spring 2016, a single 0.5 ml blood sample was collected from each individual once emergence had been observed. Immediately following sample collection using 0.5 in 25 g needles with a 1 mL syringe, samples were transferred to heparinized vials and placed on ice until sample processing within five hours of collection.

Samples were transported to a processing area, where sample vials were manually inverted 10 times, following which approximately 50  $\mu$ l whole blood was transferred into a heparinized capillary tube and centrifuged at 11,500 rpm for 5 min for the determination of packed RBC volume. The remaining whole blood sample was centrifuged for 8 min at 5000 rpm, and 50  $\mu$ l separated plasma were pipetted immediately into a disposable test tube and analyzed within 2 hr after separation, with the exception of one sampling occasion when this timeline was not possible. On this occasion, samples were centrifuged and plasma was separated and refrigerated at 0 – 4 °C until analysis seven days later. Plasma osmolality was determined using a freezing point osmometer ( $\mu$ Osmette™ Model 5004, Precision Systems Inc., MA, USA) after calibration with control standards. The remaining volume of separated plasma was transferred to cryovials and placed on ice until transported from the field to -80 °C storage. Plasma concentrations of AST, calcium, glucose, LDH, potassium, sodium, and uric acid were analyzed by IDEXX BioResearch Pathology Services (IDEXX Laboratories, Inc., ME, USA) within one year of collection.

We observed highly variable hematocrit measurements that we interpreted as indicating lymphatic fluid dilution of samples, a known complication from the chosen subcarapacial venipuncture location (Bonnet et al. 2016). Therefore, hematocrit levels were included in analyses based on the assumption that the concentrations of plasma biochemical variables would change linearly with hemodilution from lymph.

## *2.6 Soil temperature monitoring*

In order to match the shell temperature to soil temperatures to determine the timing of spring emergence, once horizontal above-ground movements had apparently ceased, a ground stake was installed in the soil at 50 – 200 cm from the turtle's hibernaculum. Each ground stake had three temperature dataloggers (iButton Thermochron DS1922L or DS1921G, Embedded Data Systems LLC, KY, USA) set at the soil surface, 7.5 cm beneath the surface, and 15 cm beneath the surface to capture the probable range of burrowing depths of overwintering *T. c. carolina* in this region (Madden 1975; Claussen *et al.* 1991). Each datalogger was held in a waterproof plastic case containing desiccant that was glued to the wooden stake at the appropriate depth. Once the stake was installed in the soil, it was surrounded by a wire mesh cage to deter animal disturbance. Dataloggers were programmed to record at 0.5 °C resolution every 40 minutes, and were subsequently downloaded approximately every 50 days. On occasions in which turtles shifted position, ground stakes were relocated so that the soil temperatures were monitored in close proximity to the hibernacula.



### *2.7 Determination of overwintering status*

Date of entry into hibernation was determined by a classification system based on light levels recorded by the shell archival data loggers. A total of 264 direct observations made over the course of the study were used to validate geolocator readings. The maximum light level recorded when a turtle was confirmed to be in its refugia and not visible above the leaf litter by the observer was substantially lower than the minimum light level recorded when a turtle was observed on the surface (Figure 1). The restrictive definition of the maximum light level observed during refugia occupancy was used as the classification threshold to ensure that no surface activity would be incorrectly classified as refugia use. Weekly tracking observations were then used to augment this classification to ensure that no above-ground surface movements were incorrectly classified as refugia occupancy. The earliest date of all-day refugia use after which no horizontal surface movements were recorded between September – December 2015 was chosen as the date of entry into hibernation for analyses.

Date of exit from hibernation was determined as the earliest of the following: (1) basking temperatures recorded by the shell data logger (shell temperatures  $> 5^{\circ}\text{C}$  above surface soil temperatures; Currylow et al. 2013), (2) direct observation by observer of the turtle above the leaf litter and active, or (3) surface-level light levels recorded by the archival light logger. We chose to identify date of exit from hibernation using any of these three indicators because the light logger was placed on the rear of the shell, and in some cases, because turtles only partially emerged headfirst from hibernacula on the first day of emergence, the light logger did not always detect such emergence events.

## 2.8 Statistical analysis

All analyses were performed using R Statistical Software v. 3.3.2 (R Core Team 2014). One male turtle was excluded from all analyses because of geolocator failure. Body condition was calculated using the scaled mass index (Peig & Green 2009), which standardizes the prediction for an individual's body condition by accounting for the scaling relationship between body mass and body size, whereas a traditional body condition index tends to be biased towards larger individuals because larger individuals will have larger absolute body components (Peig & Green 2009). The scaling exponent and coefficient for the index were determined using fall MCL and the first fall body mass measured for each individual. Males and females were combined because there were no significant differences ( $\chi^2_{(1)}=0.540$ ,  $p$ -value=0.462) in the slopes of the relationship between MCL and mass by sex. The scaling exponent was estimated to be 1.706, and the arithmetic mean value for MCL for the study population was 134.5 mm. This equation was then used to calculate an encounter-specific body condition index value for each sampling occasion for each individual.

In order to reduce dimensionality of the independent variables due to the small sample size, principal components analyses were performed on the correlation matrix of plasma biochemistry values and hematocrit levels for immergence and emergence separately. The principal components analysis for immergence included every sampling encounter. While this resulted in an uneven number of replicates per individual being included in the principal components analysis, we chose to use this approach in order to capture as much variation among the biochemical variables as

possible during fall sampling. For two individuals with missing sodium and potassium concentrations due to small sample size, we substituted the values from the other sampling occasion for that same individual. One outlier was excluded from the spring principal components analysis; this turtle exited hibernation in mid-March, but was not sampled until 27 days later and thus the plasma biochemical profile was unlikely to accurately reflect physiological condition at time of emergence. Components explaining at least 75% of the variance were then included as candidates in model selection.

For investigating the effect of candidate components on dates of immergence, we included all individuals with at least two sampling events ( $n = 15$ ) as the random effect in generalized linear mixed models using the “lme4” package (Bates et al. 2015). The dependent variable was the number of days from the sampling occasion until immergence date, *i.e.*, time to immergence, which was modeled as a Poisson distribution as appropriate for non-zero count data. Each candidate component score, sex, and body condition (scaled to a mean of zero and a range of two standard deviations from the mean) were run in separate models, and variables with  $P > 0.2$  were excluded from model comparisons. We next ran generalized linear mixed models, without interaction among the fixed effects to avoid overfitting because of the small sample size, using all possible additive combinations of candidate variables (Table 2.3), and the model with the lowest Akaike’s information criterion corrected for small sample size (AICc) was selected as the final model.

For investigating the effect of candidate components on dates of emergence, we ran generalized linear models using the “lme4” package (Bates et al. 2015). The

dependent variable was the Julian date of emergence, which was modeled as a Poisson distribution as appropriate for non-zero count data. Each candidate component score, sex, body condition (scaled to a mean of zero and a range of two standard deviations from the mean), and duration of hibernation (scaled to a mean of zero and a range of two standard deviations from the mean) were run in separate models, and variables with  $P > 0.2$  were excluded from model comparisons. We next ran generalized linear models, without interaction among the fixed effects to avoid overfitting because of the small sample size, using all possible additive combinations of candidate variables (Table 2.3), and the model with the lowest Akaike's information criterion corrected for small sample size (AICc) was selected as the final model. When one or more models were within 2 AICc values of the model with the lowest AICc, we selected the most parsimonious model as the final model.

### **3. Results**

Samples were collected from turtles in October on average 31 days (range 3 – 73 days) prior to immergence. Turtles entered into hibernation between 17 October – 4 December 2015, with half of the animals in hibernation by October 30. We collected samples from turtles on average 2 days (range 0 – 5 days) after emergence. Turtles exited from hibernation between 11 March – 9 May 2016, and the average duration of hibernation was  $170 \pm 20$  days. Excluding the 11 March outlier, exit dates ranged from 13 April – 9 May, with the average being  $171 \pm 20$  days after January 1. All plasma metabolite concentrations and osmolality values were within normal ranges for wild *T.*

*carolina* (Table 2.1; Hutton and Goodnight 1957, Kimble and Williams 2012, Adamovicz et al. 2015).

Principal component 1 ( $\beta = 0.27 \pm 0.07$ ,  $z = 3.70$ ,  $P < 0.001$ ) and principal component 5 ( $\beta = 0.17 \pm 0.05$ ,  $z = 3.43$ ,  $P < 0.001$ ) were included in the final model for time to immergence in hibernation (Tables 2.2, 2.3). Lower first principal component scores, meaning lower concentrations of calcium and sodium, as well as lower overall concentration of plasma solutes, were associated with less time to immergence. In addition, lower fifth principal component scores, meaning higher potassium, calcium, and lower hematocrit concentrations, were associated with less time to immergence (Figure 2.3). The negative loading for calcium (-0.642) in the fifth principal component score would overwhelm the positive loading for calcium (0.472) in the first principal component score, resulting in a net positive effect of calcium concentration on shorter times to immergence.

While models for timing of emergence from hibernation that included duration of hibernation or the fifth principal component were within 2 AICc values of the model with the lowest AICc, the most parsimonious model within 2 AICc values of the model with the lowest AICc was the null model (Tables 2.4, 2.5).

#### **4. Discussion**

We found evidence that physiological condition may influence certain aspects of phenological asynchrony associated with hibernation in *T. c. carolina* at our study site. We confirmed that lower concentrations of plasma solutes, indicative of good

hydration, and higher levels of calcium are associated with shorter time to immergence into hibernation. It is likely that higher levels of calcium in the fall are indicative of turtles in better condition, because females will use body reserves of calcium to shell eggs (Norris & Carr 1996) after ovulation occurs in May or June (Dodd 2001; Burke & Capitano 2011). This result corroborates the idea that behavioral plasticity enables risk-avoidance; in this case, turtles may be more likely to enter into hibernation earlier if in better physiological condition in order to decrease the risk of exposure to lethal cold temperatures, which can be a major source of mortality for *T. c. carolina* populations (Neill 1948).

We failed to find support for our prediction that turtles in better physiological condition emerge from hibernation later, but we cannot be certain that this result was not related to our methods. It may be that the relative change in plasma biochemical variables from entry into hibernation until exit from hibernation is more biologically significant than absolute concentrations in plasma measured after emergence. However, because our weekly tracking in the fall was not frequent enough to observe turtles when they were above the surface once activity levels had decreased in later October and November, we were not able to obtain blood samples for most individuals within less than five days of entry into hibernation. Turtles were not captured after they had entered into the soil to avoid disrupting the turtles' natural behavior. As a result, blood samples were likely collected too early in the season to accurately quantify physiological condition immediately prior to hibernation, and thus we cannot reliably compare immergence and emergence concentrations to obtain relative differences.

The complex relationships between various indicators of physiological condition and the timing of hibernation provide intriguing avenues for exploration into behavioral plasticity and constraints on seasonality within ectotherm populations. If physiological condition mediates the timing of immergence into hibernation, as these results indicate, then the environmental conditions in the preceding spring and summer that affect production of forage or abundance of invertebrates may be more relevant to the timing of seasonality in the fall, or even in the following spring, than previously realized. Since earlier immergence into hibernation seems to be in part a result of better physiological condition, and if this physiological condition has a carry-over effect on activity in the following spring, then the timing or size of clutches could be affected. Observed patterns in seasonality in one year may thus be more dependent on conditions in the previous year than on conditions in the observed year, representing a lag effect on physiological condition. Our sample size was too small to allow us to incorporate environmental variables in our model selection process and thus we are unable to draw inferences regarding the relative importance of environmental cues versus physiological condition on the timing of hibernation. Future work addressing the relative importance of recent physiological condition and more temporally separated environmental conditions could prove valuable for addressing fundamental ecological questions concerning risk, individual behavior, and population persistence in chelonians.

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Table 2.1. Plasma biochemical concentrations of *Terrapene carolina carolina* by sex prior to and after hibernation, in New York, USA, 2015 – 2016. Values are means  $\pm$  standard deviation.

| Season  | N  | Osmolality<br>(mOsm/kg) | Aspartate<br>aminotrans-<br>ferase ( $\mu$ L) | Glucose<br>(mg/dL) | Calcium<br>(mg/dL) | Potassium<br>(mmol/L) | Sodium<br>(mmol/L) | Lactate<br>dehydro-<br>genase ( $\mu$ L) | Uric acid<br>(mg/dL) |
|---------|----|-------------------------|-----------------------------------------------|--------------------|--------------------|-----------------------|--------------------|------------------------------------------|----------------------|
| Males   |    |                         |                                               |                    |                    |                       |                    |                                          |                      |
| Fall    | 9  | 291.8 $\pm$ 19.4        | 61.0 $\pm$ 30.0                               | 31.5 $\pm$ 17.0    | 11.4 $\pm$ 2.2     | 3.0 $\pm$ 0.4         | 127.4 $\pm$ 4.8    | 201.1 $\pm$ 142.7                        | 1.32 $\pm$ 0.6       |
| Spring  | 9  | 289.8 $\pm$ 14.8        | 68.9 $\pm$ 31.2                               | 69.7 $\pm$ 45.8    | 9.6 $\pm$ 0.8      | 3.3 $\pm$ 0.6         | 125.8 $\pm$ 4.5    | 589.9 $\pm$ 699.5                        | 4.1 $\pm$ 2.7        |
| Females |    |                         |                                               |                    |                    |                       |                    |                                          |                      |
| Fall    | 10 | 296.8 $\pm$ 12.2        | 41.2 $\pm$ 25.9                               | 20.2 $\pm$ 11.9    | 15.2 $\pm$ 2.4     | 2.9 $\pm$ 0.4         | 130.0 $\pm$ 3.1    | 159.2 $\pm$ 78.4                         | 1.06 $\pm$ 0.4       |
| Spring  | 10 | 286.5 $\pm$ 10.3        | 39.0 $\pm$ 13.4                               | 44.4 $\pm$ 16.5    | 12.0 $\pm$ 2.1     | 2.7 $\pm$ 0.3         | 129.2 $\pm$ 3.9    | 275.0 $\pm$ 76.9                         | 2.8 $\pm$ 2.0        |

Table 2.2. Principal components analysis of plasma biochemical profiles of *Terrapene carolina carolina* in New York, USA, October 2015. The first five components were used in variable selection for modeling time to immergence in hibernation. Loadings  $\geq 0.4$  in the first five components are in bold.

|                               | Comp. 1      | Comp. 2       | Comp. 3       | Comp. 4       | Comp. 5       | Comp. 6       | Comp. 7       | Comp. 8      | Comp. 9       |
|-------------------------------|--------------|---------------|---------------|---------------|---------------|---------------|---------------|--------------|---------------|
| Osmolality                    | <b>0.445</b> |               | -0.161        | 0.264         | -0.214        | <b>0.660</b>  | -0.345        | -0.298       |               |
| Aspartate<br>aminotransferase | -0.363       | <b>-0.500</b> | -0.252        |               |               |               | 0.226         | -0.183       | <b>0.683</b>  |
| Glucose                       | 0.177        | <b>-0.544</b> |               | <b>-0.461</b> |               |               | <b>-0.493</b> | <b>0.450</b> |               |
| Calcium                       | <b>0.472</b> |               |               | <b>0.429</b>  | <b>-0.642</b> | -0.228        | 0.322         | <b>0.508</b> | 0.246         |
| Potassium                     | -0.277       | 0.259         | <b>-0.408</b> | -0.361        | <b>-0.462</b> | <b>0.403</b>  | 0.199         | 0.371        |               |
| Sodium                        | <b>0.405</b> | -0.243        | 0.212         | <b>-0.488</b> | -0.129        |               | <b>0.582</b>  | -0.341       | -.135         |
| Lactate<br>dehydrogenase      | -0.260       | <b>-0.562</b> |               | <b>0.400</b>  |               | 0.123         | 0.170         | 0.100        | <b>-0.628</b> |
| Uric acid                     | 0.180        |               | <b>-0.662</b> |               | -0.263        | <b>-0.546</b> | -0.158        | -0.303       | -0.206        |
| Hematocrit                    | 0.275        |               | <b>-0.502</b> |               | <b>0.725</b>  | 0.169         | 0.227         | 0.248        |               |

Table 2.3. Model selection using the corrected version of Akaike's information criterion (AICc) to explain time to immergence in hibernation for *Terrapene carolina carolina* in New York, USA, 2015. All fitted candidate models are listed. "Component X" refers to the indicated principal component from the principal components analysis of plasma biochemical parameters.

| No. | Model                                      | AICc   | $\Delta$ AICc | AICc weight | <i>K</i> | <i>LL</i> |
|-----|--------------------------------------------|--------|---------------|-------------|----------|-----------|
| 1.  | Component 1 + Component 5                  | 293.75 | 0.00          | 0.75        | 4        | -142.16   |
| 2.  | Body condition + Component 1 + Component 5 | 296.01 | 2.27          | 0.24        | 5        | -141.90   |
| 3.  | Component 1                                | 303.11 | 9.36          | 0.01        | 3        | -148.14   |
| 4.  | Body condition + Component 1               | 303.13 | 9.38          | 0.01        | 4        | -146.85   |
| 5.  | Component 5                                | 307.87 | 14.12         | 0.00        | 3        | -150.52   |
| 6.  | Body condition + Component 5               | 308.90 | 15.15         | 0.00        | 4        | -149.74   |
| 7.  | Body condition                             | 311.24 | 17.50         | 0.00        | 3        | -152.21   |
| 8.  | Intercept                                  | 311.82 | 18.07         | 0.00        | 2        | -153.71   |

Table 2.4. Principal components analysis of plasma biochemical profiles of *Terrapene carolina carolina* in New York, USA, spring 2016. The first five components were used in variable selection. Loadings  $\geq 0.4$  in the first five components are in bold.

|                               | Comp. 1       | Comp. 2      | Comp. 3       | Comp. 4       | Comp. 5       | Comp. 6 | Comp. 7 | Comp. 8 | Comp. 9 |
|-------------------------------|---------------|--------------|---------------|---------------|---------------|---------|---------|---------|---------|
| Osmolality                    | 0.288         | 0.385        | <b>0.443</b>  | -0.372        | -0.150        | -0.235  | 0.261   | -0.519  | 0.136   |
| Aspartate<br>aminotransferase | <b>-0.543</b> | -0.117       | 0.196         | -0.301        | 0.151         |         |         | 0.145   | 0.719   |
| Glucose                       |               | 0.122        | <b>0.571</b>  | 0.179         | <b>0.658</b>  | 0.236   | 0.191   | 0.231   | -0.209  |
| Calcium                       | 0.282         |              | <b>-0.474</b> | <b>-0.516</b> | 0.212         | 0.200   | 0.532   | 0.248   |         |
| Potassium                     | -0.383        | 0.286        | 0.176         |               | <b>-0.579</b> | 0.197   | 0.447   | 0.331   | -0.220  |
| Sodium                        | <b>0.441</b>  | -0.190       | 0.348         | -0.296        | -0.290        | -0.116  | -0.326  | 0.596   |         |
| Lactate<br>dehydrogenase      | <b>-0.426</b> | -0.250       |               | <b>-0.533</b> | 0.116         | -0.255  | -0.102  |         | -0.606  |
| Uric acid                     |               | <b>0.530</b> | -0.203        |               | 0.206         | -0.703  |         | 0.349   |         |
| Hematocrit                    |               | <b>0.601</b> | -0.110        | -0.289        |               | 0.486   | -0.543  |         |         |



Table 2.5. Model selection using the corrected version of Akaike’s information criterion (AICc) to explain time to emergence from hibernation for *Terrapene carolina carolina* in New York, USA, 2016. All fitted candidate models are listed. “Component X” refers to the indicated principal component from the principal components analysis of plasma biochemical parameters.

| No. | Model                                 | AICc   | $\Delta$ AICc | AICc weight | <i>K</i> | <i>LL</i> |
|-----|---------------------------------------|--------|---------------|-------------|----------|-----------|
| 1.  | Duration of hibernation               | 129.63 | 0.00          | 0.35        | 2        | -62.42    |
| 2.  | Component 5                           | 130.10 | 0.47          | 0.28        | 2        | -62.65    |
| 3.  | Duration of hibernation + Component 5 | 130.50 | 0.87          | 0.23        | 3        | -61.39    |
| 4.  | Intercept                             | 131.48 | 1.85          | 0.14        | 1        | -64.61    |

Figure 2.1. Validated light levels of microhabitats of *Terrapene carolina carolina* in New York, USA, in 2015 – 2016.

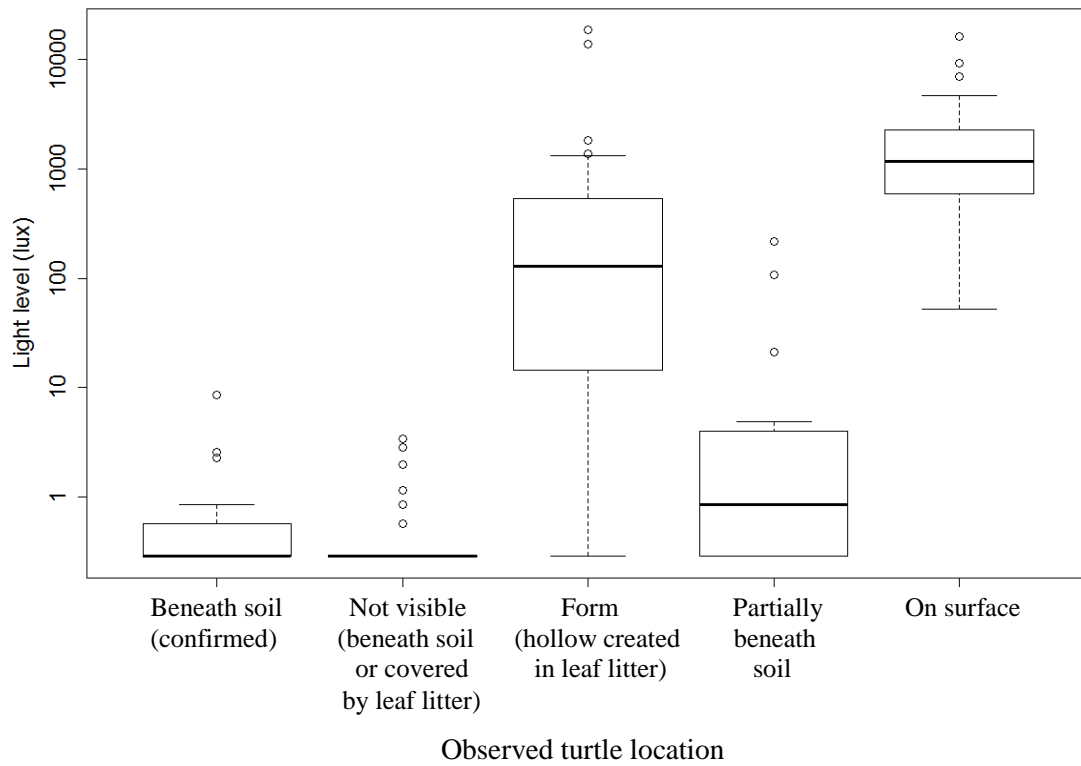


Figure 2.2. Marginal model of days to immergence in hibernation by the fixed effect of first principal component score (positive loadings of calcium, sodium, and osmolality) of *Terrapene carolina carolina* in New York, USA, in October 2015. The covariate, the fifth principal component, is held at its mean of zero. Gray shading indicates 95% confidence region.

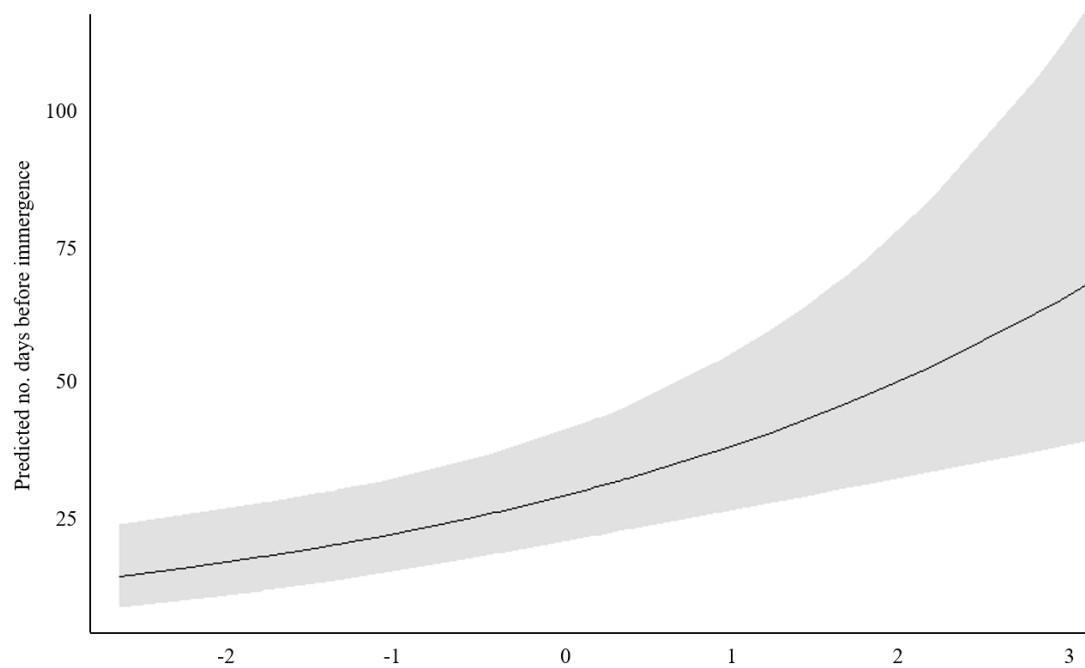
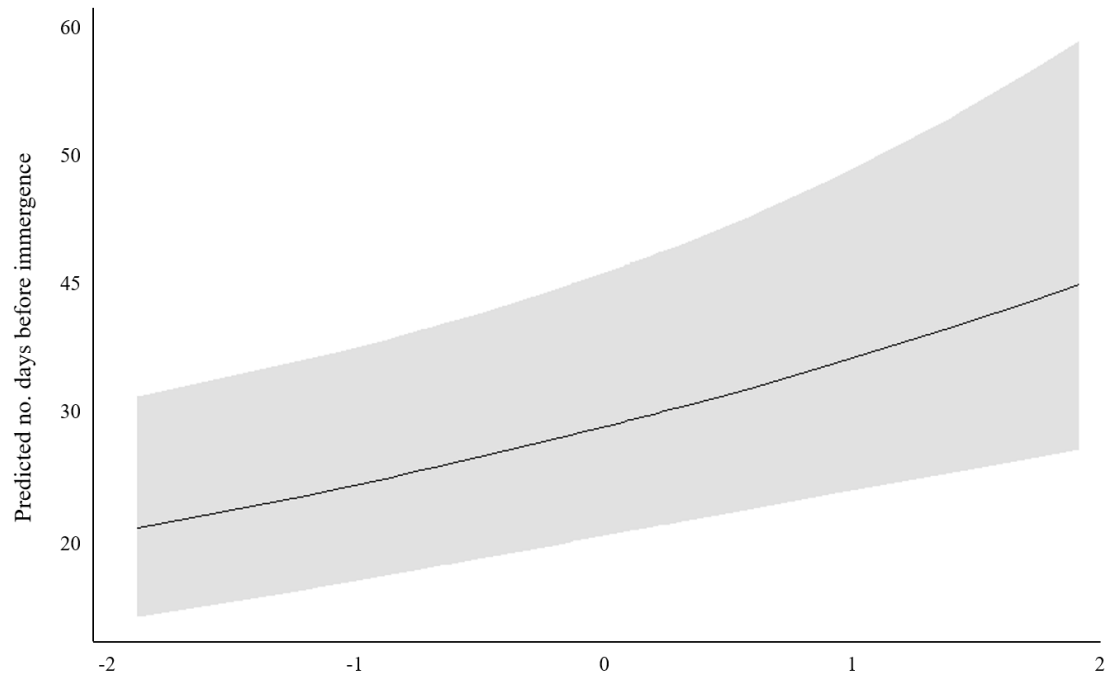


Figure 2.3. Marginal model of days to immergence in hibernation by the fixed effect of fifth principal component score (positive loading of hematocrit, negative loadings of potassium and calcium) of *Terrapene carolina carolina* in New York, USA, in October 2015. The covariate, the first principal component, is held at its mean of zero. Gray shading indicates 95% confidence region.



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